IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	Examiner: Fronda, Christian L.
Woon-Lam Susan LEUNG, *et al.*	)	Art Unit: 1652
Application Serial No. 09/422,528	)	Confirmation No. 5652
Filed: October 21, 1999	)	Attorney's Docket No. GNE-0128A
For: **PROCESS FOR BACTERIAL PRODUCTION OF POLYPEPTIDES**	)	Customer No. 35489

FILED VIA EFS – AUGUST 17, 2009

DECLARATION OF MICHAEL W. LAIRD, Ph. D.

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

I MICHAEL W. LAIRD, Ph.D. declare and say as follows: -

1. I obtained a B.S. in Biology from Iowa State University in 1991, a Ph.D. in Microbiology from Arizona State University in 1996, and completed postdoctoral training at Genentech, Inc. (CA) in 1999. Subsequently, I was employed by Human Genome Sciences with increasing levels of responsibilities and titles until 2005.

2. In 2005 I joined Genentech, Inc. as Senior Scientist where my current title is Associate Director/Late Stage Cell Culture.

3. I have extensive experience with cell culture processes for the recombinant production of heterologous polypeptides, including therapeutic antibodies and growth factors, in various host organisms, including bacterial hosts, in particular various *Escherichia coli* (*E. coli*) strains.

4. My Scientific Curriculum Vitae is enclosed as Exhibit A and forms part of this Declaration.

5. I have read and understand the disclosure and claims of the above-identified patent application.

6. I have also read and understand the Office Actions mailed on March 17, 2008 and February 17, 2009 in connection with the above-identified patent application and Applicants' response of December 17, 2008, and the following publications, cited in the Office Actions:

Hart et al., BIO/TECHNOLOGY Vol. 12, November 1994;

EP 0 155 189 (Wetzel et al.); and

Dien et al., Appl Environ Microbiol. 1997 May; 63(5):1689-95.

7. Claims 1-13 and 15-25 pending in this application concern a process for recovering refractile particles containing a heterologous polypeptide from bacterial periplasm in which the polypeptide is insoluble by coordinated expression of nucleic acid encoding the desired heterologous polypeptide and nucleic acid encoding a phage lysozyme, thereby releasing insoluble refractile particles from the peptidoglycan layer of the bacterial periplasm. Claim 1 states that "*expression of the nucleic acid encoding the phage lysozyme is induced by the addition of an inducer after about 50% or more of the heterologous polypeptide has accumulated.*" An example of the heterologous polypepides produced by the claimed process is human insulin-like growth factor-I (IGF-I), an exemplary lysozyme is T4 phage lysozyme and an exemplary inducer is arabinose.

8. I understand the patent Examiner's position to be that the invention claimed in this application is obvious over the combination of references listed in paragraph 6 of this Declaration, even though the references do not teach or suggest to wait until 50% or more of the human IGF-I has accumulated before inducing expression of the phage lysozyme. I further understand that the reason for this conclusion is that, according to the Examiner, one of ordinary skill in the art would have been motivated to wait until 50% or more of the human IGF-I or other representative heterologous protein has accumulated before inducing expression of T4 phage

lysozyme in order to obtain a greater yield of the heterologous protein, and would have had a reasonable expectation of success to achieve this goal.

9. The timing for the induction of the phage lysozyme is a very delicate balance between guaranteeing that the cells have achieved an appropriate concentration of the recombinant product prior to lysozyme production and ensuring that there is enough lysozyme at harvest so that its effectiveness is not severely diminished. It was known in the art at the time the present invention was made that high level production of recombinant proteins during fermentations is growth associated and occurs at its maximum rate during the exponential growth phase of the culture (Kim and Ryu, Biotechnol Bioeng, 38:1271-1279 (1991) (Exhibit B) and Gupta et al., J Biotechnol 68:125-134 (1999) (Exhibit C)). As bacterial cultures enter into stationary phase upon nutrient and energy depletion, the machinery responsible for recombinant protein production is depleted in favor of maintaining the cellular metabolic activity (i.e. eliminates non-essential activities to maintain viability) (Martin, Mol Microbiol 5:3-10, (1991) (Exhibit D); and Dedhia et al., Biotechnol Bioeng, 53:379-386 (1996) (Exhibit E)). The process disclosed and claimed in the present application requires the production of two recombinant proteins: human IGF-I and a phage lysozyme. Before the invention disclosed and claimed in this application it was not known whether after accumulation of 50% or more of human IGF-I, when the nutrient and the energy pools are depleted to the point where cell survival is primary, the remaining cellular machinery would be able to produce sufficient amounts of the second recombinant protein, the phage lysozyme, to be effective to perform its intended role. Therefore, while waiting until accumulation of at least 50% of human IGF-I might have been desirable in order to increase IGF-I production, it was unpredictable whether this measure would not defeat the purpose of the process as a whole, which requires the production of the phage lysozyme in an amount sufficient to release retractile particles containing IGF-I from the cellular matrix or cell wall.

10. Based on my experience with the expression of heterologous proteins in bacterial hosts, including *E. coli*, and in view of the explanation provided in paragraph 9 of this Declaration, it is my considered scientific opinion that at the time the present invention was made one of ordinary skill in the art would not have had a reasonable expectation that at the end

of a long fermentation process and after substantial IGF-I product accumulation the host could produce enough of the phage lysozyme to be effective.

11. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further, that these statements are made with the knowledge that willful statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent granted thereon.

Dated: 17 Aug 09

MICHAEL W. LAIRD, Ph.D.

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